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13. ABSTRACT ***(Maximum 200 Words)***

The purpose of this project was to develop a reliable, non-invasive technique to gain access to the lining of the milk ducts. In this contract we have devised a technique to reliably identify all of the ductal orifices and confirmed their anatomy in a normal woman using MRI to correlate the ductal orifices to the ducts. The second accomplishment was to demonstrate that we can reliably retrieve ductal epithelial cells as washings by cannulating a duct with a double lumen catheter. Finally, we confirmed the ability to identify a specific ductal orifice, cannulate it with the double lumen catheter and obtain washings in six women scheduled for surgery. This work has led to two patents and 510k approval of the catheter from the FDA. It is being developed by a venture-backed company, Pro•Duct Health Inc, and is in multicenter clinical trials leading to a commercial product in the spring of 2000. The catheter and procedure of ductal lavage will enable clinicians and researchers to non invasively and repeatedly sample the ductal epithelial cells in high risk women, opening the way for the identification of markers, and also provide the ability to monitor the success of chemoprevention techniques. In addition, the intraductal approach to the breast can be exploited for local application of ablative or gene therapy.

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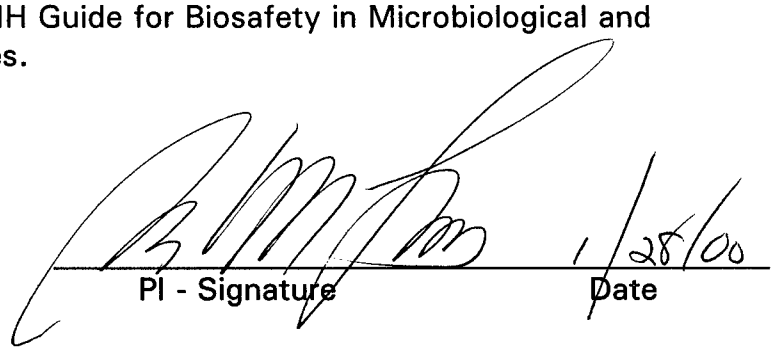

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INTRODUCTION

The goal of this project was to develop a reliable, non-invasive technique to gain access to the lining of the milk ducts. In a previous IDEA grant we demonstrated the feasibility of an intraductal approach to breast disease, the ability to obtain ductal cells through washings, and the general anatomy of the nipple duct orifices and ductal systems. In this contract we continued the project, confirming our previous anatomical studies *in vitro* and *in vivo*. We also refined and tested the double lumen catheter as a means of lavaging breast ducts and retrieving diagnostic cells. This work has led to two patents and a start up medical device company dedicated to commercializing the intraductal approach to the breast.

BODY

By the time the contract was awarded, some of the work described in the application had been completed. Accordingly, a revised scope of work was developed and approved in January 1997.

Revised Statement of Work DAMD17-96-C-6117
1/31/97

Phase IA: Anatomy of nipple duct orifices
Part one. Anatomy of nipple duct orifices in lactating women
data analysis of nipple data (completed year 1)
Part two. Anatomy of the breast duct orifices and their identification (completed year 2)
Product: a general map of the nipple duct orifices (completed year 2)

Phase IB: Anatomy of the ductal systems
Part one: archival ductograms
analysis of data (completed year 1)
Part two: cadaveric studies
refining of technique of cannulation of nipple duct orifices with double lumen catheter (completed years 2 and 3)
multi duct ductograms followed immediately by corrosion cast preparations (unsuccessful)
analysis of data
Product: general map of the ductal system (completed year 3)

Phase II: Cannulation of the ducts
developing a technique to identify all of the nipple duct orifices in a woman (completed year 2)
cannulation of surgical patients (completed year 3)
Product: reliable method for cannulating all breast ducts in one breast or a particular breast duct (completed year 3)

Phase III: Obtaining cells/tissue
refinement of the double lumen catheter to insure cell retrieval in the detatched fresh mastectomy breast (completed year 2)
cannulation of surgical patients and collection of cells and tissue (completed year 3)
Product: a technique for obtaining tissue from breast ducts (completed year 3)

Phase IA: Anatomy of nipple duct orifices
Part one. Anatomy of nipple duct orifices in lactating women

Data analysis of nipple data (completed year 1) *note the original work was done under DOD Grant DAMD17-94-J-4281 and the analysis completed under this contract. Some of this analysis was also reported in the final report of DOD Grant DAMD17-94-J-4281*

Background: On gross inspection, it is not easy to identify the nipple duct orifices in a non-lactating woman. However the different openings are readily apparent in a lactating woman. We therefore decided to inspect the nipples of lactating women and try to map their nipple duct orifices. We hypothesized that there is a standard number of nipple duct orifices and a general pattern. As with other aspects of anatomy, we recognized that there would be individual variations but we felt that by observing a sufficient number of women we could begin to discern this pattern.

After obtaining permission from UCLA's Human Subjects Protection Committee, we approached La Leche League (a breast feeding support group) nationally and locally and a local breast feeding support network located at the "Pump Station" a commercial source of breast pumps. We asked lactating women if we could observe their nipples and map their nipple duct orifices. Initially we had planned to photograph the orifices, but it was immediately obvious that this was not possible. Milk exits the breast through different orifices but very soon pools on the surface of the nipple obscuring the individual openings. The observer needed to rapidly assess the number and location of orifices before pooling occurred. This approach tended to underestimate the number of orifices since it is more difficult to count a large number of orifices than a few. The same observer mapped all of the women in an effort to minimize observer bias. Immediately after observing the nipple the researcher diagrammed the orifices on a prepared grid (Figure 1, Appendix A). The location of the orifices was relationally characterized since the position of the woman and her breast was not perfectly standardized. Nonetheless, the resulting observations gave us a crude estimation of the usual number of nipple duct orifices and their approximate location.

Results: Data were collected on 424 nipples and 219 women. The mean number of nipple duct orifices was 5 with a range of 1-17. The patterns were symmetrical; 98.8% of women had 13 or less orifices. Figure 2 (Appendix A) is a two-way histogram showing the relative frequency/numbers of the nipple openings by location. Darker shading indicates higher relative numbers in that region of the nipple. The most common location was in the center of the nipple. We performed a K-means cluster analysis[1] to determine empirically the number of nipple orifices and their pattern. The nipple openings grouped into 13 clusters. Figure 3 (Appendix A) shows the mean location and the standard error of the mean location, on the coordinates defined as x and y of the nipple openings in each of the 13 empirical clusters. In an attempt to determine whether two adjacent clusters were distinct we analyzed pairs of nipple duct orifices ie how often a woman had cluster 1 if she had cluster 2. Table 1 (Appendix A) gives the tabulation of 786 pairs of nipple openings by the empirical cluster.

Almost every woman had a duct orifice in the center of her nipple (cluster 2) and a second one in the central area but oriented to the upper outer quadrant (cluster 11). The overall pattern could be described variably but the most consistent finding was 4-5 central orifices: one

central with two upper and two lower. The upper and lower orifices could be two each or one each, with the second orifice located further peripherally than the first. In addition, there could be additional orifices in the lower inner and upper outer areas. This pattern is consistent with the tear shape of the breast.

Applying this insight to our work with cadavers, we confirmed that we could almost always find the orifices in the center of the nipple and rarely peripherally. This pattern was then correlated to the ductogram analysis.

Part two. Anatomy of the breast duct orifices and their identification (completed year 2 and 3)
Further anatomy data was collected by observing the orifices which yielded fluid in a nipple aspirate fluid study (NAF study, see Table 2, Appendix A). In this study, the nipple was first dekeratinized and then a woman in the upright seated position was asked to massage her breast(s). A suction device was then applied to nipple and suction withdrawn to 10 cc while the woman continued the massage. The procedure was halted once fluid was identified from any of the milk ducts or after three attempts. The nipple duct orifices of all of the women studied were graphed by a single observer on the nipple grid (Figure 1, Appendix A). Of 55 women analyzed, the nipple duct orifices confirmed the previously identified pattern with the most consistency being in the central ducts and less in the peripheral ones. It is possible, of course, that central ducts are more likely to yield fluid with this technique.

Product: a map of the nipple duct orifices (completed year 3)
A general map of the nipple duct orifices was constructed and is included in Appendix A as Figure 4.
Most important, however, was the concept of the central ductal orifices and the peripheral ones. This information was used in our preoperative study to identify the orifice which would lead to the area of the breast seen on mammogram to contain an abnormality. (See Phase III study.)

Phase IB: Anatomy of the ductal systems
Part one: Ductogram Study
Note the original work was done under DOD Grant DAMD17-94-J-4281 and the analysis completed under this contract. Some of this analysis was also reported in the final report of DOD Grant DAMD17-94-J-4281

Background: We had obtained 1312 archival ductograms from studies done by Otto Sartorius in the 1960's. These ductograms represented 656 distinct ducts (two cross sectional views of each duct) in 470 women. Dr. Sartorius had studied many asymptomatic women without nipple discharge. The ductograms had been analyzed under Dr. Sartorius' tutilege into ten categories based on observation. We elected to reexamine the ductograms using a different method to confirm the Sartorius designations.[2]

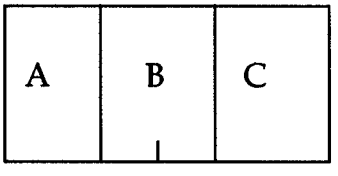
There are many biases in this analysis. First of all, there was no attempt to catheterize a particular duct but rather whichever one was most apparent. The resulting ductograms will then favor the more obvious ductal orifices. These probably correlate with the central orifices we noted in the nipple duct orifice study (see above). It is possible that some of the ductal systems are not represented in this series at all. Secondly there was no standardization of technique. The ductograms were all done by the same technician but there was no standardization of positioning

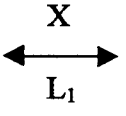
of the breast. This means that a ductal system which appears to be lateral in one woman could actually be lower in another. Taking this into account, we still felt that an anatomical analysis of this series of ductograms would give us a general idea of the pattern and number of ductal systems. This could then be correlated to the nipple duct orifice study.

The Sartorius categories are depicted in Figure 5 (Appendix A) with the frequencies noted in Table 3 (Appendix A). The approximate location of the center of the duct on the chest wall and the volume of the duct was determined for each ductogram by first measuring the areas in the region of the duct to either side in the two dimensional photograph. A planimeter was used to outline the extent of the duct in relation to the total area and the areas on each side. The nipple was not included in the measurements. The back wall of the breast was defined as the rib cage, muscle or edge of the ductogram. The back wall was chosen which would yield the smallest area while still including the entire duct. For example, if the ductogram showed both the muscle and the rib cage the muscle would be the back wall if it enclosed the entire duct. If the duct extended past the muscle wall, the rib cage would be used as the back wall. When the ductogram didn't include the muscle or rib cage (i.e., didn't include the whole breast on the film) the total breast measurement was inaccurate.

In the analysis we assumed that the breast is a hemisphere, the cross sections are semi circles and the ductal shape is triangular. We divided the semicircular cross-section into three sections. B is the section of the ductogram. A is the section lateral to the ductogram and C is the section medial to the ductogram. Using A, B and C from each cross-sectional view (measured quantities) we can define and calculate:

X duct = location of the center of the duct, side to side (medial lateral) view
Y duct = location of the center of the duct head to foot (inferior superior) view
L_1, L_2 = length of duct respective views
V = volume of duct = function of L_1, L_2





Using the measured quantities A B and C from each cross sectional view where the total area in that view of the breast is standardized to one, we define
xduct = A_{ML}+0.5 B_{ML} to be the center of the duct in the medial lateral view, yduct = A_{IS} +0.5B_{IS} to be the center of the duct in the inferior-superior view, and the relative volume of the duct to be B_{ML}*B_{IS}

Figure 5 (Appendix A) is a two-way histogram showing the relative frequency/concentration of the ducts, by ductal center location (xduct, yduct). Darker shading indicates higher relative numbers of ducts projected in that region of the chest wall.

Figure 6 (Appendix A) shows the mean location and the standard error of the mean location, on the coordinates defined by xduct and yduct, of the ducts in each Sartorius category. Table 4 gives this information in tabular form.

For those ductograms with age of the patient known (n=400, range 14 to 88 years of age, median age 40 years), we analyzed the relationship between age and xduct, yduct, and volr. Significant ($p<0.01$) negative correlations between age and yduct and between age and volr, and a significant positive ($p<0.05$) correlation between age and xduct, were found. The significant negative correlation between age and volr persisted when controlling for the effects of xduct and yduct upon volr through a multiple linear regression analysis. Similar correlations were found when we repeated the analysis, chosing a single ductogram per woman (n=286 with age known) to avoid the technical problem of within-subject correlation.

2-D histograms.

Cluster analysis. We performed a K-means cluster analysis[1] to determine empirically the number of ductal systems and their characteristics. We clustered (grouped) the ductograms/individual ducts based on the calculated chest-wall location of their centers (xduct, yduct).

Pairs. Because our categorizations are tentative due to the inherent "noise" of the data, we considered the question of whether a duct in one woman classified into one category is the equivalent of a classification into an adjacent category in another; that is, they represent the same ductal system. To explore this question, we considered pairs of ducts within a woman for all women who had more than one duct catheterized, and tabulated the pairs by the two categories represented per pair. Thus, for example, if no woman or very few had a pair of ducts in categories A and B, where A and B are physically close, then we might conclude that A and B represent the same ductal system. Conversely, if many women had pairs in categories C and D, or within a category, then we have evidence that there is more than one distinct ductal system in these categories.

Results: The results for the ductograms were messy. Using all 656 ductograms, there was no convergence of the clustering algorithm into fewer than 20 clusters. Using one ductogram per woman (selected at random) resulted in 11 clusters of the 474 ductograms. Figure 7 (Appendix A) shows the mean location and its standard error of each of these clusters. Table 5 (appendix) gives the cross-classification of these ductograms into these empirical clusters and the Sartorius categories. Using only ductograms from women of age 50 and under (304 ductograms for n = 220 women) gave 13 clusters. Figure 8 (Appendix A) shows the mean location and its standard error of each of these clusters. Table 6 (Appendix A) gives the cross-classification of these ductograms into these empirical clusters and the Sartorius categories. Table 7 (Appendix A) gives the tabulation of 202 pairs of ducts (for all women) by the Sartorius category of each member of the pair. The Sartorius categories and the nipple openings clusters representing ducts located in the center of the breast have multiple pairs, whereas women tend not to have pairs of ducts in the outer regions.

The general pattern of ducts match Sartorius' analysis with a central duct which goes directly back from the nipple. This is surrounded by a central upper duct, a central lower duct, a central medial duct and a central lateral duct. This grouping forms the central circle of duct orifices. More peripherally are the upper lateral and upper medial ducts, the lower lateral and lower medial ducts and the deep lateral duct. The deep duct describes a pattern where a narrow duct travels towards the chest wall and then arborizes in the upper outer quadrant. In picturing

these ducts it is important to remember that the breast is not two-dimensional as we have depicted it previously. The ducts do not extend in a radial fashion from the nipple but rather they travel back from the nipple toward the chest wall. One way to visualize the ducts is to picture each orifice as a flashlight aimed toward the chest wall. The beam of light on the chest wall represents the area of that ductal system.

Although not an exact match, the nipple duct study and the ductogram study do corroborate certain findings. There is a central collection of ducts and ductal orifices and a more peripheral one - concentric circles rather than pieces of pie. This schematic is useful but shouldn't be considered representational. The limitations of these two observational studies have been noted. Our next step should be to confirm our findings *in vivo*. Nonetheless, the orientation of the ductal systems make an accurate surgical approach even more difficult.

Other interesting findings include the fact that the ductal systems become narrower and longer with age. This is undoubtedly a result of ptosis which increases with age and is reflected on the mammograms.

Part two. Confirming the anatomy/cadaveric studies
Background: Although the analysis of archival single ductograms has been invaluable in determining the pattern of the ducts, it has not been able to show us the interaction between ductal systems. For this aspect we employed cadaver breasts. We tried to confirm the data derived from Phase IB with sequential multi-duct contrast ductograms of fresh cadaveric mastectomy specimens

Materials and methods: Cadavers were purchased through the UCLA Willed Body Program. Bilateral radical mastectomies were performed on the freshly thawed cadavers. The breast specimen included the overlying skin (to sternum, clavicle, rectus fascia and latissimus dorsi muscle) and pectoralis muscles. Sutures are used to orient the specimen. The breast specimens were refrozen and stored at -10 degreees C until several specimens had been accumulated. The breast specimens were partially thawed before use. Sequential ductograms on the specimen were obtained after harvest as follows. Duct orifices were identified with the aid of a dissecting microscope with a 20x-80x magnification. All identified ducts were cannulated with a commercial galactography kit (Taber-Rothschild Galactography Kit #M030895-13, Manan Medical Products Inc, Northbrook IL) or a .014" guide wire (Boston Scientific). A double lumen catheter was then threaded over the guide wire into the duct. After fixation of all of the catheters into place with a suture, each catheter was sequentially injected with 0.20-0.50 cc of renograffin contrast followed by radiographs taken in the AP view. The duct was then be washed out with saline. This sequence was repeated for each duct which was cannulated.

Results: In our series of cadavers, our ability to blindly catheterize ductal orifices was variable. In some cases we had catheterized a sebaceous cyst rather than a duct. In other cases there was extravasation of renograffin . It was not clear whether this was a consequence of using cadaver breasts or whether they had been ruptured in the process of doing the cannulation. We were able to conclude that the ductal systems are separate. We also noted occasional cysts in continuity with the ductal systems (a finding previously described by Sartorius).

Casting
Materials and methods: Following the imaging studies, we tried leaving the cannulas in place for corrosion casting. A methylmethacrylate resin (Batson's No. 17 Plastic Replica and Corrosion Kit, Polysciences, Warrington PA) was used for casting of the ductal system. The solution consists of a base, a catalyst and a promoter with an additional methylmethacrylate monomer added to reduce viscosity as necessary. The volume of contrast needed to fill the duct was estimated from the volume of contrast that was needed to fill the duct as seen on the ductogram, approximately 0.2-0.5cc. Each duct was injected with a resin containing a different coloring agent (color pigment, Batson's anatomical corrosion Kit, Polysciences). After injection the breast was allowed to polymerize overnight at 25 degrees. The tissue surrounding the ducts was corroded with 30% potassium hydroxide over several months at 50 degrees. Any remaining fibrous tissue was carefully dissected away.

Results: This procedure was not successful. It took several months to corrode the surrounding tissue with potassium hydroxide. In our initial efforts, part of one duct was casted with evidence of extravasation distally. A second attempt was completely unsuccessful. We were unable to obtain more cadaver breasts due to a change in policy in the morgue.

Product: On the basis of these studies, an artist's rendition of the anatomy of the breast ducts was developed and is included in Appendix A as Figure 9.

Phase II: Cannulation of the ducts
Developing a technique to identify all of the nipple duct orifices in a woman (completed year 2)
Background: Although our previous analysis of the anatomical patterns and general number of ducts was useful information, it did not help us in an individual case. Knowing that most women have 13 or fewer ductal orifices doesn't help us when confronted with an individual woman. We still needed a technique which would mark all of the nipple duct orifices in any one woman. We explored several approaches to this problem, including: (1) consumption of an inert colored material which might color the nipple duct fluid and demonstrate the nipple. Although cows who eat certain materials can color their milk, they are lactating. Non-lactating women may not have any fluid and therefore coloration would not be useful. (2) Applying a colored liquid such as betadine and applying suction to the nipple with the hope that the release of suction would suck the colored liquid into the nipple orifice. This was tried on several cadavers without success, probably because of the keratin plug in the nipple duct orifice noted below. (3) Stimulation of nipple duct fluid with oxytocin, a material used in stimulating breast feeding. One of the investigators tried a nasal spray of oxytocin and noted no increase in breast ductal fluid.

None of these was successful. We decided to approach the nipple directly. One nipple areolar complex was removed from a cadaver and fixed. Over a thousand oriented slices were made and stained. On one slide we were able to identify a ductal system extending directly to the nipple duct orifice. The orifice was found to have a keratin plug. There was no transitional epithelial as demonstrated by a low molecular weight keratin antibody which stains ductal epithelial cells and not squamous cells. Based on these data we performed more experiments:

a) Dekeratinization and application of fluorescent antibodies (non-viable)
Our ability to selectively stain the ductal glandular epithelium led us to hypothesize that we could selectively stain the ductal epithelium and then treat it with a fluorescent antibody which would be visible at the nipple surface. This would achieve our task of identifying the nipple duct orifices. In the first year we developed this technique in a step-wise fashion employing pig nipples (a readily available hairless animal model). We developed a dekeratinization technique using acetic acid which removed the keratin layer without damage to the epithelial tissue. We then attempted to develop a technique for specifically fluorescing the ducts using a primary antibody of mouse anti-human epithelial membrane antigen (EMA) and a secondary antibody of goat anti mouse IgG (H and L chains) FITC conjugate. To visualize the FITC probe, we used a xenon lamp (150W) with a blue light filter (488nm), argon laser (protective) goggles (514nm). This fluorescent procedure only resulted in diffuse fluorescence which was demonstrated to be due to diffuse nonspecific binding.

b) Natural Fluorescence (non-viable)
While looking for fluorescence caused by the antibodies noted above we observed a natural fluorescence of the nipple. We were able to demonstrate that nipple aspirate fluid also exhibited fluorescence and explored whether this natural fluorescence could be used to identify ducal orifices. Multiple observations of women and men demonstrated that the fluorescence was secondary to keratin and not specific for the breast duct orifices.

c) Nitrocelullose filters (non-viable)
Imayama et al[3] reported the application of nitrocelulose filters for 24 hours to the nipples of women who had had breast cancer. Subsequent analysis of the filter paper demonstrated increased levels of CEA. This suggested that there is an imperceptible leakage of fluid from the nipple over time. We collected nitrocellulose filters from a number of volunteers with the hope that we could capitalize on this leakage to develop a map of the orifices on the filter paper. Although we were able to confirm Imayama's finding of evidence of nipple aspirate fluid, there was also transfer of keratin to the paper. We were not able to identify a consistent marker which would make this a viable approach.

d) Transareolar dye injection (viable)
This method is based on several observations that were made while attempting to identify and map the duct orifice. The key observations were procured during practice and histologic analysis of specimens used in the earlier approaches.

The ducts are designed to secrete and deliver products to the surface of the nipple. The natural design of the mammary ductal system hinders the opposing direction with a keratin plug that occludes the ductal orifice(s) and protruding surface epithelium that acts as an incomplete valve. At the base of the nipple there are lactiferous sinuses that taper as they course to the surface of the nipple. This approach uses the natural wicking of the lactiferous sinuses toward the surface of the nipple to identify the ductal orifices.

Method:
a.) 1-2 cc's of lymphazurin, a small molecular dye, is introduced transareolarly into the base of the nipple with a syringe. By random diffusion the dye enters the ductal lumens of the lactiferous sinuses.

b.) The tapering nature of the ductal lumens as they course to the surface wicks the dye to the surface of the nipple via capillary action.
c.) The ductal orifice(s) are identified at the surface of the nipple as deep blue pinpoints within 10-20 seconds with all appearing within 2-3 minutes.
d.) The potential ductal orifices are then cannulated with a guidewire or cannula and then a single or double lumen catheter
e.) Tissue specific marking dyes are then instilled into the ducts
f.) The breast is processed for histology and serial horizontal sections under the nipple are taken to demonstrate the duct profiles. Ducts successfully identified and cannulated would display ink within the ductal lumen.

Results: This method was attempted in a series of 13 detached breasts (fresh breasts which had just been surgically removed) (Appendix A, Table 8). Allowing for a learning curve, we were able to identify all of the ducts and confirm them, resulting in the constant and reproducible identification of each and every duct. Water-resistant dye of both the same color and different colors support several conclusions about ductal anatomy/nipple anatomy:

1) there are between 5 and 9 ducts/nipple
2) the ductal systems are separate and non-anastomosing
3) serial sections of the nipple confirmed that our technique identifies all ductal orifices
4) histology demonstrated that the dye can reach the far recesses of the ductal lobular unit

Confirmation *in vivo*. The procedure was repeated in a woman (PI) using lymphazurin mixed with lidocaine. The procedure was well tolerated and eight ductal orifices were identified and cannulated without difficulty.

MRI: using the dye injection technique in a volunteer (PI), MRI ductograms were done on four ducts in one breast. Gadolinium was used for three of the studies and proved to diffuse out of the ductal system very rapidly. The fourth study was done with conray and was more successful. This study demonstrated several findings:

- The duct orifices correlated to the ductal systems in the same area of the breast; i.e., the orifice at twelve o'clock corresponded to the ductal system that was located in the upper portion of the breast.
- There were no inter-duct connections
- Gadolinium diffused out of the ducts quickly

Cannulation of surgical patients (completed year 3)
See section below on attached breasts and Phase III Chilean study
Product: reliable method for cannulating all breast ducts in one breast or a particular breast duct (completed year 3)

Phase III: Obtaining cells/tissue
Refinement of the double lumen catheter to insure cell retrieval in the detached fresh mastectomy breast (completed year 2)

Cannulation of the duct orifices
Part 1: Detached breasts
Background: In our IDEA grant we devised a double lumen catheter which would allow a continuous flow of saline throughout the ductal system. The prototype catheter was a 3 French double lumen catheter. The proximal lumen is smaller in diameter and is used for instilling saline; the distal lumen is larger and is used for aspiration. We initially studied detached breasts at UCLA (series 3000), or breasts which had just been removed surgically but not yet sent to pathology. We assumed that these fresh breasts would still have intraductal cells that could be retrieved through washings. We studied 24 breasts from 12/12/96-12/17/98. These breasts were examined in the pathology work room. Ductal orifices were identified under magnification using a galactography probe when necessary. After priming the catheter, an 0.14" guidewire was then passed into the duct and the double lumen catheter was passed over the wire for a distance of about 1 cm. The guide wire was removed. Approximately 10 cc of saline was instilled through the narrower lumen and gentle suction was used to retrieve the fluid from the duct.

Results: Of the 24 breasts examined we were able to cannulate at least one duct in 18 (75%). In total 28 ducts were cannulated and washed with a yield of ductal cells in 13 (46%) and cells of any type (including degenerate cells and inflammatory cells) in 18 (64%) (see UCLA series 3000, Table 9 in Appendix A). Since we did not necessarily cannulate the duct which we thought corresponded to the pathology, we made no attempt to correlate our washing findings with the ultimate pathology.

Part 2: Attached Breasts
The next phase was to use one of these prototype catheters in "attached breasts" prior to mastectomy to confirm that we could get viable cells through the distal lumen of the catheter. After general anesthesia had been obtained, the patient's breast was prepped and draped. Mild suction was be applied to the nipple to try and elicit discharge. A dissecting microscope or loupes was used to magnify the nipple. A map was made of the orifices, which have been identified. Starting with the most promising orifice (i.e., most amount of discharge, largest) we attempted to cannulate it using either a standard set of metal dilators (galactography set by Mahan), a very small glide wire (type used in angiography). Once the duct had been cannulated and dilated to approximately 0.7-1.0 cm, the double lumen catheter was threaded into the duct. Saline was instilled setting up a continuous flow until 10cc had been collected. This procedure took approximately 15 minutes. If we were unable to complete the procedure within the 15-minute limit, we stopped prematurely. The washings were then sent to cytology for analysis.

Results: We studied 28 breasts (see Olive View series 4000, Table 9 in Appendix A). We were able to successfully cannulate at least one duct in 17 (60%). This percentage is lower than that seen in the detached breasts in part due to the time constraints in patients under general anesthesia. Of the 20 ducts successfully washed 19 (95%) had evaluable cells of any kind and 15

(75%) had ductal cells. Of the cases with ductal cells, two demonstrated malignant cells consistent with the pathology (one invasive and one DCIS).

When comparing these data with our previous results using either the ductoscope or galactography kit (single lumen), the double lumen catheter yielded more cells (60% ductoscope, 53% galactography, 77% double lumen) and more ductal cells (50% ductoscope, 29% galactography, 58% double lumen) than either previous technique. This yield was highest in the attached breasts (75% versus 46%) as would be expected (Table 10, Appendix A).

Part 3: Cannulation of surgical patients and collection of cells and tissue (completed year 3)

The final demonstration of the anatomy and cannulation hypothesis was to be a study of women who had microcalcifications and were scheduled for surgery. The plan was to use the double lumen catheter prior to surgery to cannulate the duct containing the microcalcifications and obtain a confirmatory ductogram and washings. The ductogram would confirm our ability to apply the anatomical findings and accurately identify the correct duct, and the washings would be correlated with the final pathology. Unfortunately the institutional review board at UCLA determined the double lumen catheter to be a risk to the patients and would not allow it to be used. An attempt was made to proceed with the study using a metal galactography single lumen cannula. Three women were enrolled. In one, no ducts could be cannulated. In the other two, there was perforation of the duct in question. Neither woman had any untoward sequelae but it was determined that it would be safer to do the study with the flexible double lumen catheter in another location. Therefore, a pilot study was designed and approved in Santiago Chile in conjunction with Pro•Duct Health Inc (financial support).

The study was approved by the National Cancer Institute of Chile. Six women scheduled for surgery for mammographically detected lesions were identified. After informed consent had been obtained (in Spanish), the breast was prepped and draped in a standard sterile fashion. The nipple was anesthetized using 0.5% lidocaine. The mammogram was studied and the lesion was localized to be in a particular radial sector of the breast. Subsequently it was determined to be in the central group of ductal orifices or the peripheral ones. These categorizations were used to identify the likely site of the appropriate nipple duct orifice. The nipple was then injected with lymphazurin in that sector and the orifice was identified by seeing the dye emerge from the orifice. This orifice was then cannulated first by a dilator (Manan Galactography Kit) and then by a glide wire. The double lumen catheter was then passed over the glide wire into the duct for a distance of less than 1 cm. One cc of half per cent lidocaine was instilled into the duct to further anesthetize it and then 10 cc of saline were instilled into the duct and while suction was being held on the outflow tract. The fluid was centrifuged and put into cytolyte.

Results: Six women were studied with no untoward events (Table 11, Appendix A). Seven breasts were catheterized. Of the seven ducts that were washed, four (57%) had confirmation by ductogram that the correct duct had been cannulated. In one there was no dye in the breast but some had spilled onto the patient. In another the wrong duct was cannulated and a second was attempted without success. Finally, in one women no ducts were cannulated, probably because she had an inverted nipple. Cytology of the washes correlated completely with the final pathology, with three cases of benign ductal epithelium and one demonstrating malignant cells. It should be noted that three of the four successful cannulations were in the last two women, hinting at a learning curve for this procedure.

Product: A technique for obtaining tissue from breast ducts (completed year 3)
Two patents have been filed for the double lumen catheter, the method of obtaining cells and the method for identifying the nipple duct orifices. (See Appendix B). In addition, a venture backed company was formed to commercialize this technique, Pro•Duct Health Inc. a portion of the last years work was done with additional funding from Pro•Duct Health Inc. The catheter has been improved and is now in a multi-center clinical trial to test its validity in comparison to NAF for the detection of premalignant cells in high risk women. The FDA has granted a 510 K for the catheter and a commercial product is expected on the market by spring of the year 2000.

KEY RESEARCH ACCOMPLISHMENTS

- Determination of the number and pattern of nipple duct orifices
- Determination of the pattern of ducts in the breast
- Development of a method for cannulating breast ducts and retrieving ductal epithelial cells through a double lumen catheter

REPORTABLE OUTCOMES

1. Abstracts:
   (1) The Anatomy of the Breast Ducts. Love SM* Barsky S, Chou J, Offodile R, Alpaugh M, Grosser S, Pesicka D. Presented at the San Antonio Breast Cancer Symposium, December 9-12, 1999
   (2) Identification of Premalignant and Malignant Breast Cells in High-Risk Women by Ductal Lavage: Results from a pilot study. Submitted to American Society of Breast Surgeons
2. Presentations:
   (1) Istituto Europeo di Oncologia (Milan) Grand Rounds, "Intraductal approach to the breast," July 7, 1999.
   (2) Yale University, Cancer Center Grand Rounds "An intraductal approach to the breast," September 28, 1999
3. Manuscripts: The Anatomy of the Breast Ductal System (in preparation)
4. Patents:
   (1) Methods and kits for identifying ductal orifices in a nipple
   (2) Method and kit for obtaining fluids and cellular material from breast ducts
5. 510 k approval of catheter from the FDA
6. Pro•Duct Health, Inc: a venture capital based medical device startup company commercializing this research (originally incorporated as Windy Hill Technology, see Appendix B).

CONCLUSIONS

We have demonstrated the anatomy of the breast ducts and demonstrated the ability to lavage a duct and retrieve diagnostic cells. This contract lays the foundation for a new approach to research and diagnosis of breast diseases. By reexamining the anatomy of the nipple duct orifices and the ductal anatomy, it has established a baseline that can be used to inform surgery and future research. The ability to cannulate the breast ducts lifts a past constraint to our understanding of the breast, the inability to easily and non- invasively examine the ductal epithelium and environment. It will now be possible to follow the ductal epithelium of high risk women and look for molecular markers. It will open the door for studies of the metabolism of breast duct fluid in non-lactating women.

Further work:

Although this work is being commercialized, there are still many unanswered questions. It will be important to know how often a woman can be lavaged; in other words, how long does it take for the epithelium to repopulate the duct. Once this is determined, it will be possible to see if this procedure can be used as a monitoring tool for women on chemoprevention or hormone replacement therapy. This technique may well allow future research into the molecular changes which occur in the development of breast cancer and could potentially lead to the identification of markers. Finally, this technique opens the door to the application of local approaches to therapy such as gene therapy and local ablation.

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[1] Hartigan JA. Clustering Algorithms. NY: Wiley, 1975:90-91.
[2] Sartorius OW, Smith HS, Morris P, Benedict D, Friesen L. Cytologic evaluation of breast fluid in the detection of breast disease. Journal of the National Cancer Institute 59(4), 1977:1073-1078.
[3] Imayama S, Mori M, Ueo H, Nanbara S, Adachi Y, Mimori K, Shimozono Y, Hori Y Sugimachi K, Presence of elevated carcinoembryonic antigen on absorbent disks applied to nipple area of breast carcinoma patients. Cancer 78(6) 1996:1229-1234.

Figure 1. Nipple Grid/Template

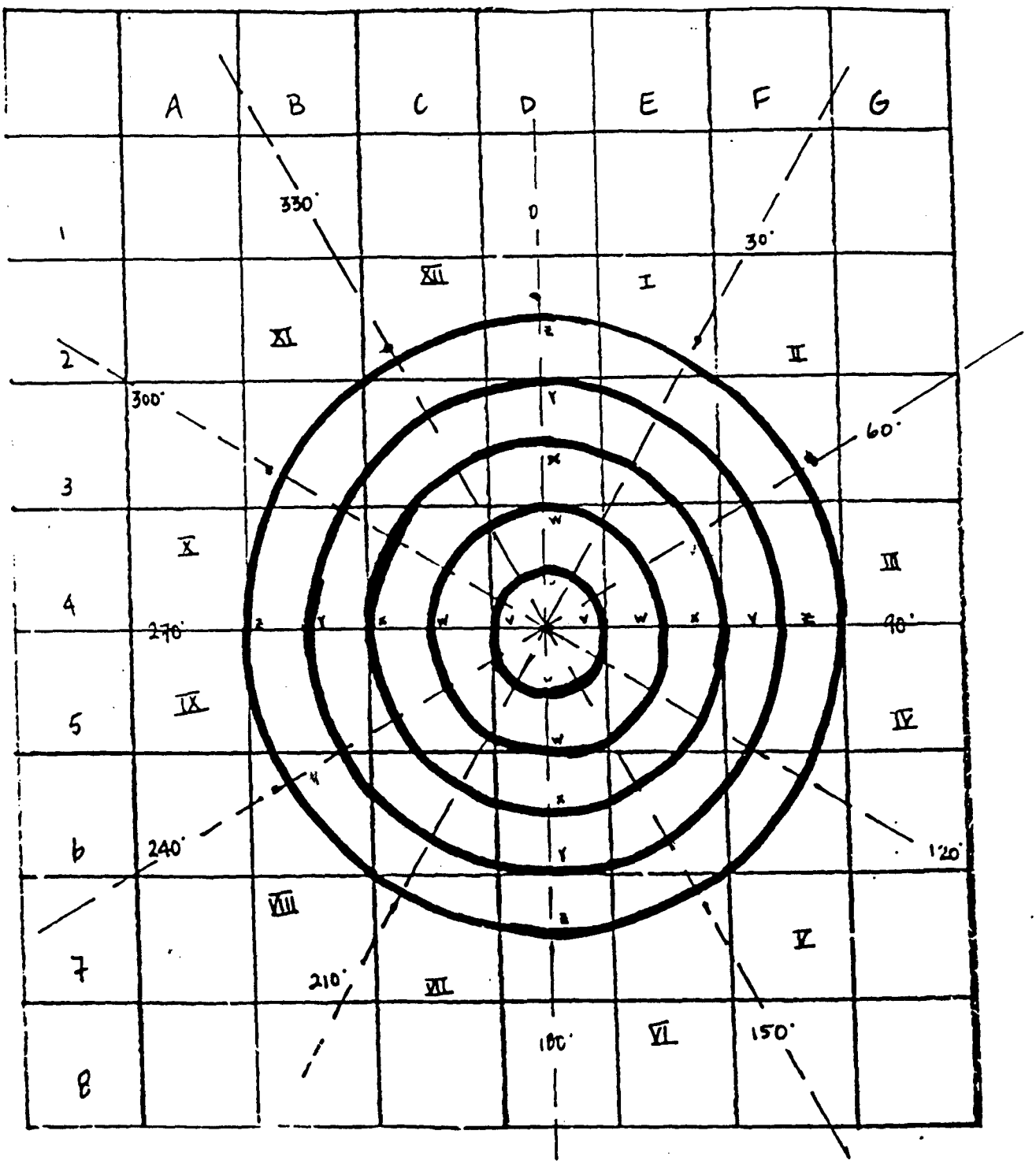

Figure 2. Two-way histogram

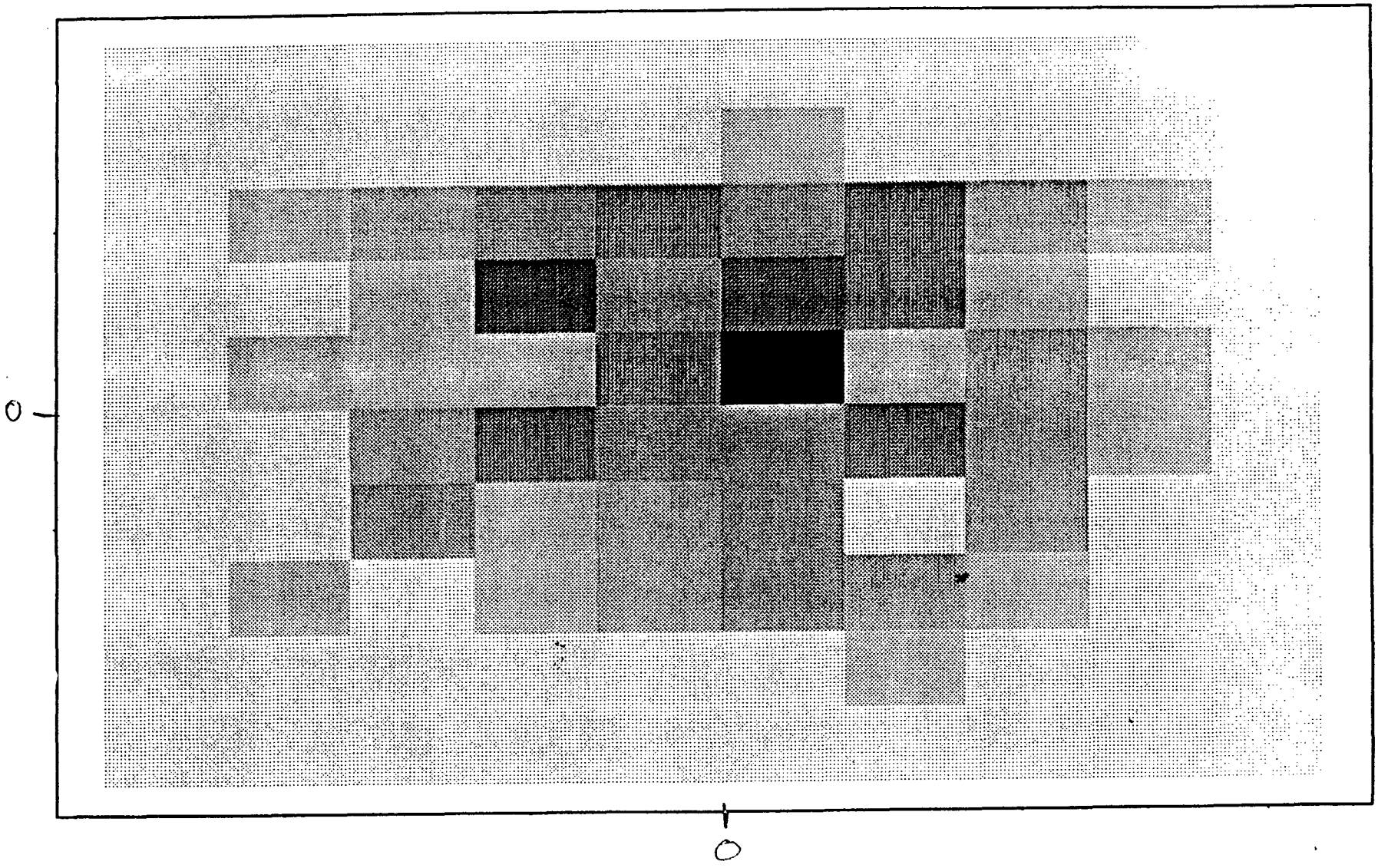

Figure 3. Mean location of nipple openings

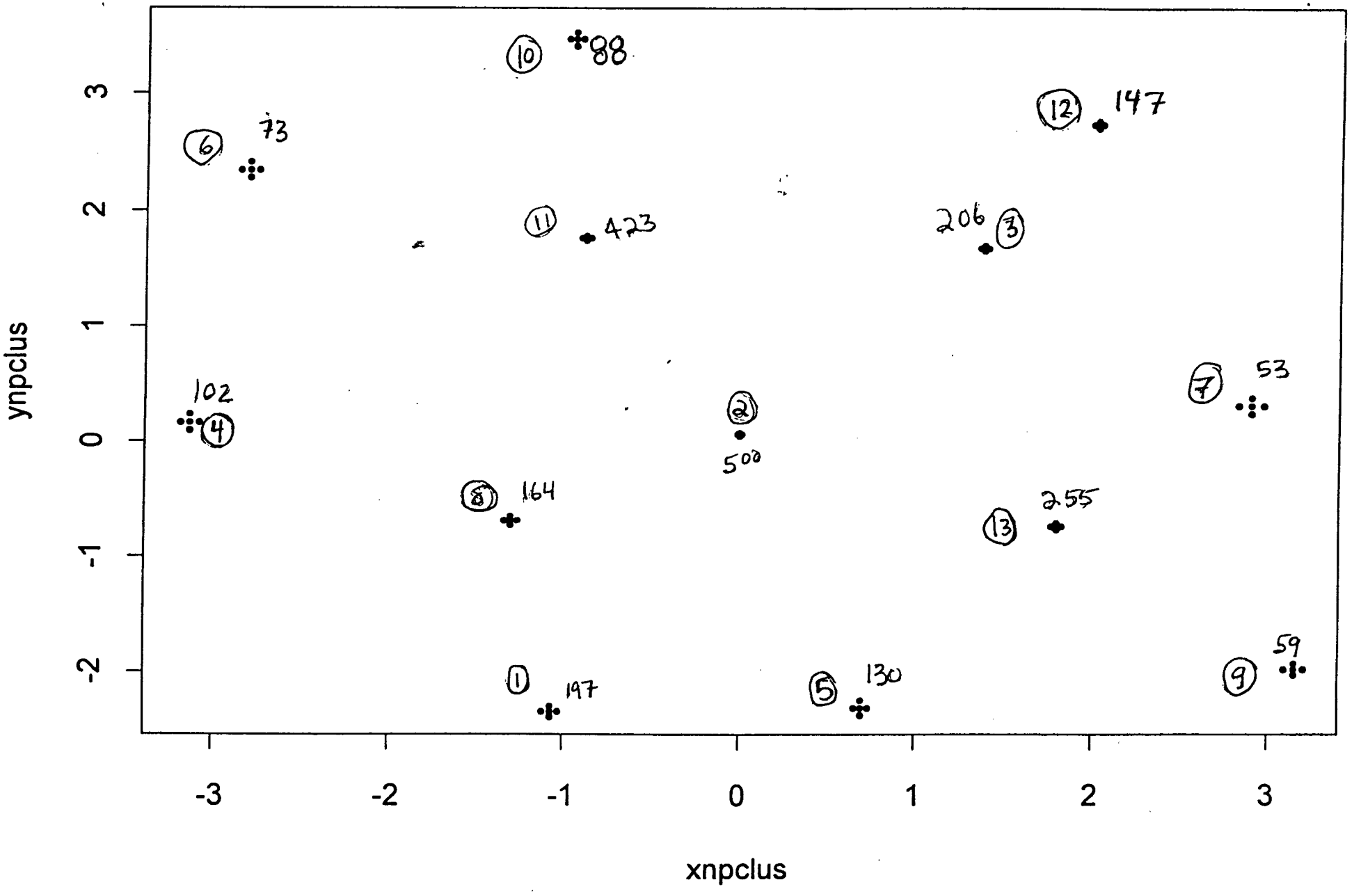

Figure 4. Map of nipple duct orifices

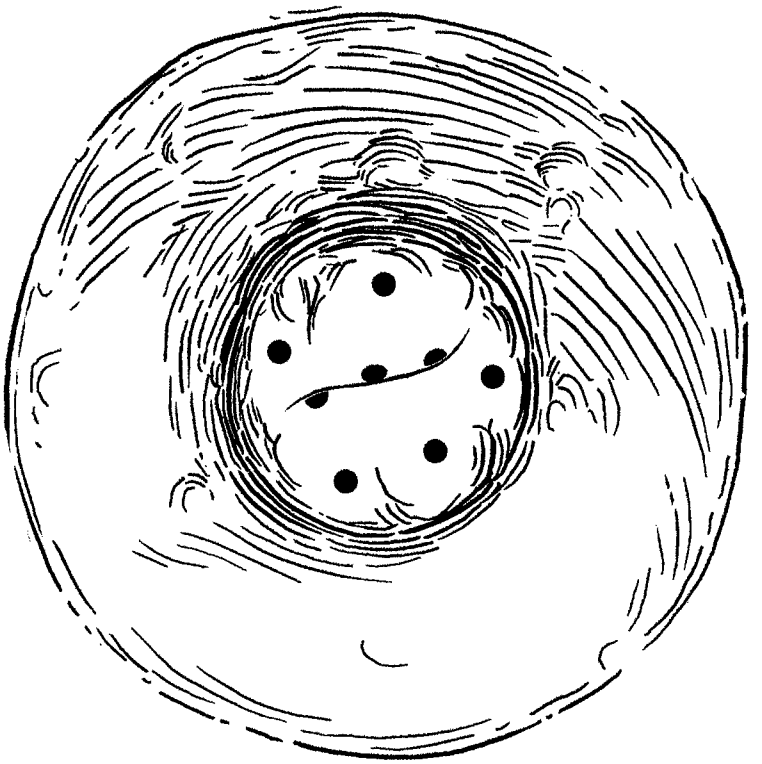

Figure 5. Two-way histogram of ductal centers (lactating women)

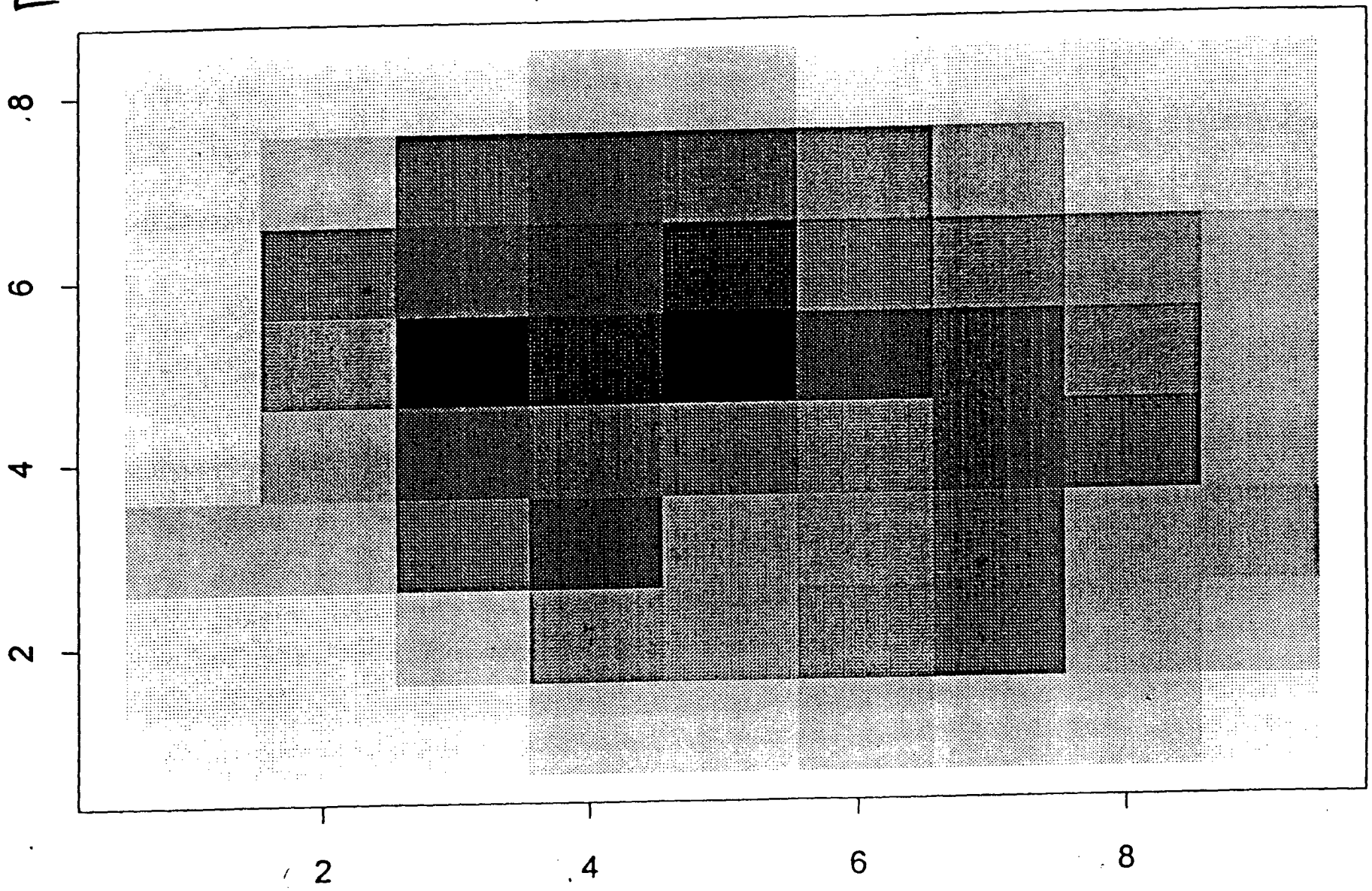

Figure 6. Mean location of the ducts/Sartorius categories

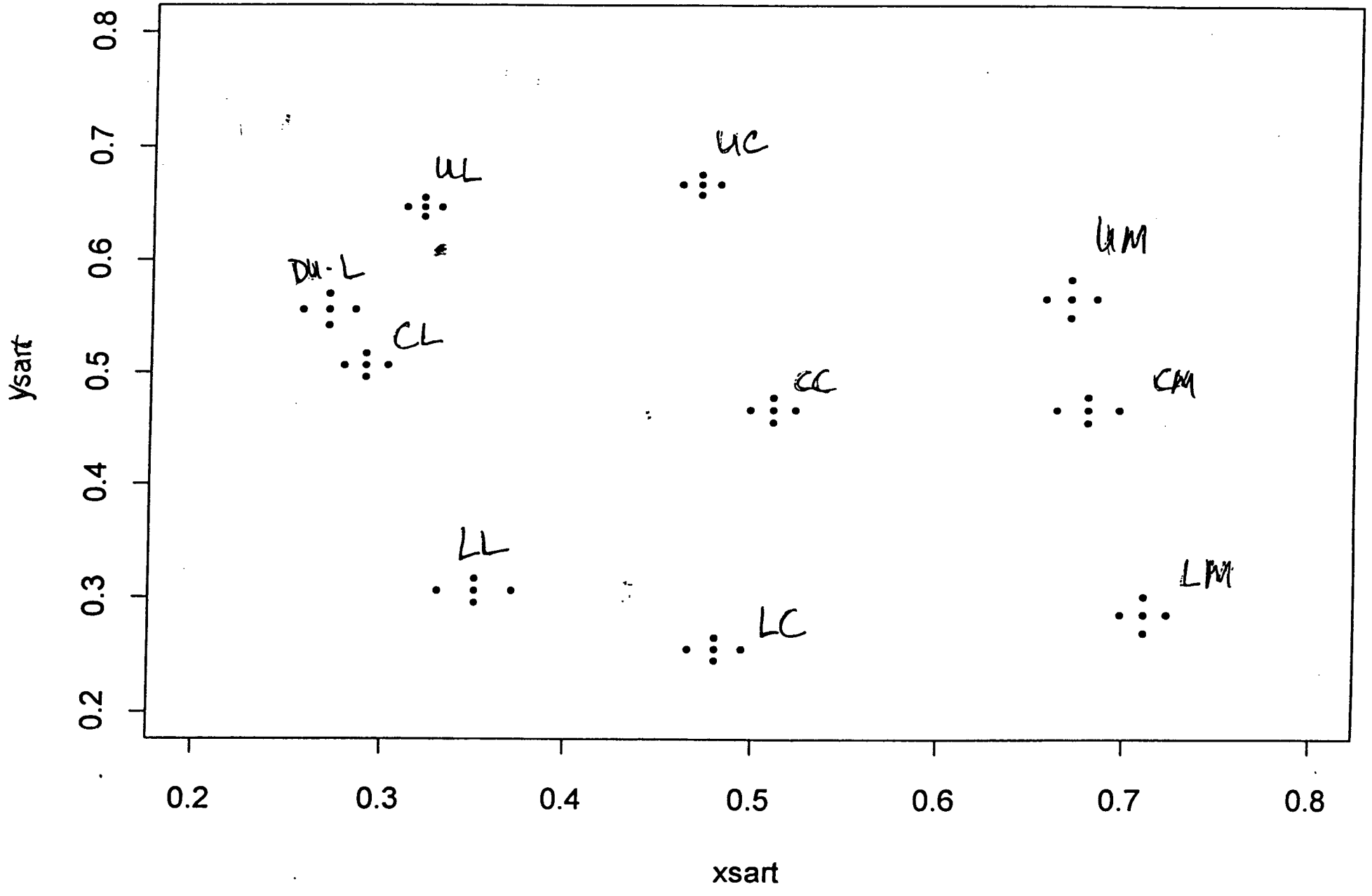

Figure 7. Mean location of ducts in empirical clusters
one ductogram/woman

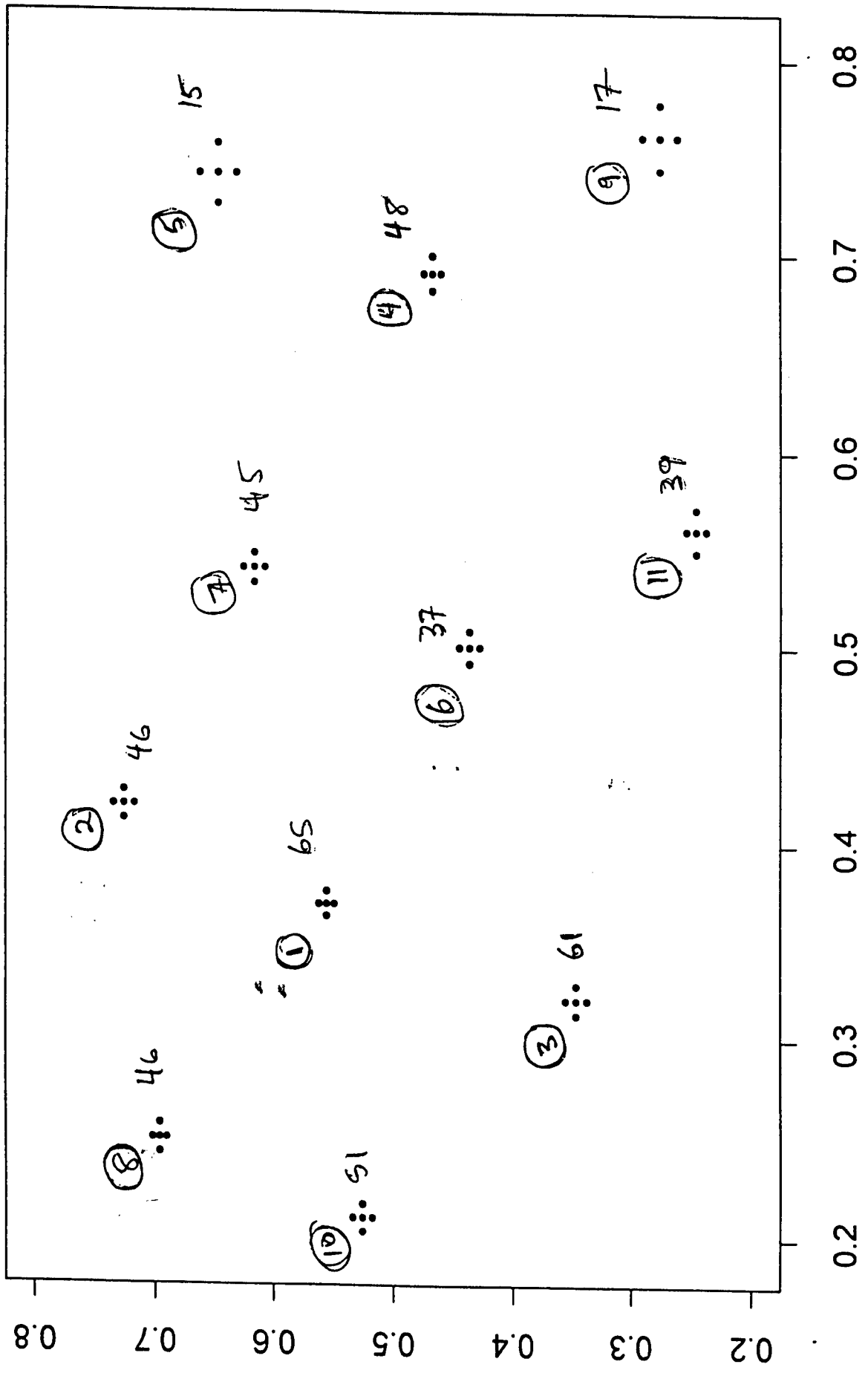

Figure 8. Mean location of ducts in empirical clusters

women < 50 years of age

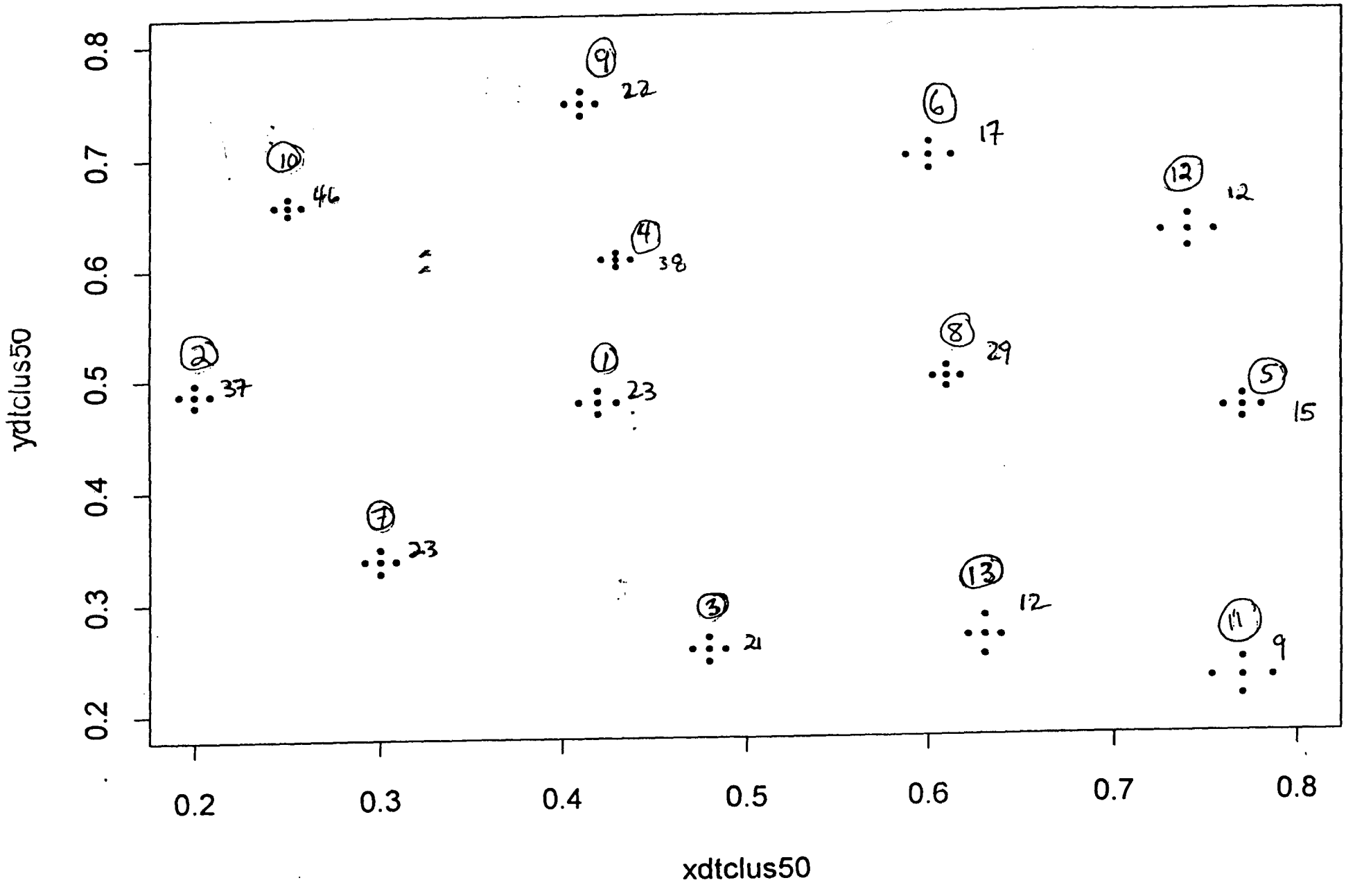

Figure 9. Rendition of nipple ducts

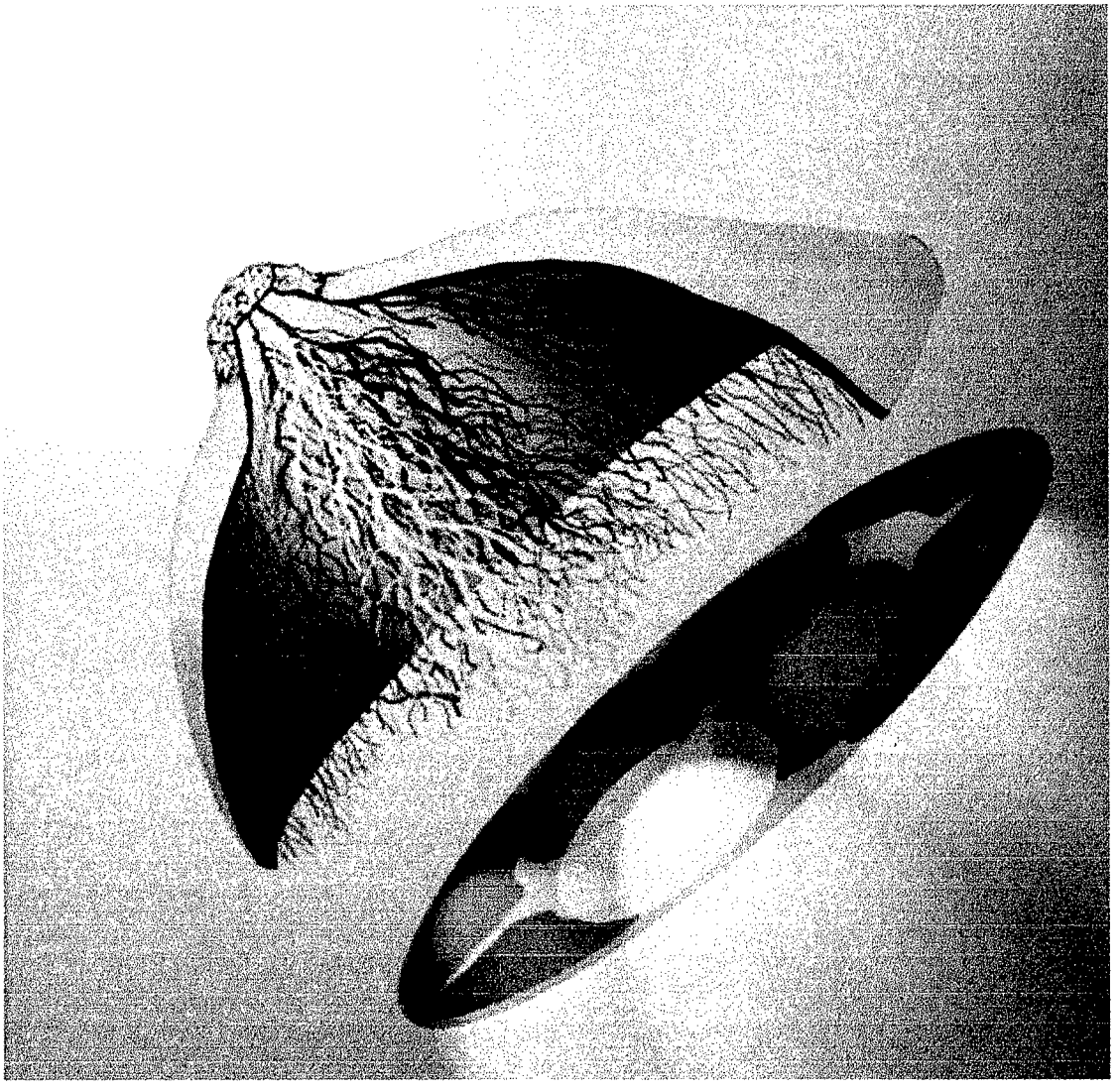

Table 1. Cross-tabulation of nipple pairs by empirical cluster

CLUSTER CLUSTER-1

Frequency	1	2	3	4	5	6	7	8	9	10	11	12	13	To
1	47	0	0	0	0	0	0	0	0	0	0	0	0	
2	247	237	0	0	0	0	0	0	0	0	0	0	0	
3	113	236	48	0	0	0	0	0	0	0	0	0	0	
4	59	137	46	14	0	0	0	0	0	0	0	0	0	
5	77	152	58	46	18	0	0	0	0	0	0	0	0	
6	45	76	45	23	31	9	0	0	0	0	0	0	0	
7	29	53	29	17	19	7	2	0	0	0	0	0	0	
8	90	199	92	39	43	29	22	28	0	0	0	0	0	
9	38	66	21	16	27	19	11	31	4	0	0	0	0	
10	56	102	41	31	38	28	14	42	26	8	0	0	0	
11	211	517	193	113	172	95	68	171	73	111	265	0	0	
12	95	178	69	37	61	33	14	65	28	58	192	33	0	
13	130	323	146	61	95	54	33	104	41	76	306	114	70	
Total	1237	2276	788	397	504	274	164	441	172	253	763	147	70	

Table 2. Frequency of nipple orifices in NAF study

	b	c	d	e	f	total
3	0	0	3	0	0	3
4	0	7	4	7	1	19
5	1	11	39	13	1	65
6	2	3	10	3	1	19
7	1	1	4	2	0	8
8	0	2	2	2	0	6
total	4	24	62	27	3	120

Table 3. Frequencies of Sartorius categories

SART	Frequency	Percent	Cumulative Frequency	Cumulative Percent
CC	82	12.5	82	12.5
CL	106	16.2	188	28.7
CM	62	9.5	250	38.1
DU-L	42	6.4	292	44.5
LC	49	7.5	341	52.0
LL	34	5.2	375	57.2
LM	33	5.0	408	62.2
UC	99	15.1	507	77.3
UL	114	17.4	621	94.7
UM	35	5.3	656	100.0

Table 4 Mean location and standard error of the mean location of the ducts in each of the Sartorius categories.

Sartorius Category	X mean (std dev)	Y mean (std dev)
CC	0.51 (0.11)	0.47 (0.10)
CL	0.30 (0.12)	0.51 (0.11)
CM	0.68 (0.13)	0.47 (0.09)
DU-L	0.27 (0.09)	0.56 (0.09)
LC	0.48 (0.10)	0.26 (0.07)
LL	0.35 (0.12)	0.31 (0.06)
LM	0.71 (0.07)	0.29 (0.09)
UC	0.47 (0.10)	0.67 (0.09)
UL	0.32 (0.10)	0.65 (0.09)
UM	0.67 (0.08)	0.57 (0.10)

SART CLUSTER(Cluster)

Table 5. Cross-classification into empirical clusters & Sartorius categories, one ductogram/woman

SART	1	2	3	4	5	6	7	8	9	10	11	Total
CC	9	2	7	10	1	20	12	0	1	0	5	67
	1.91	0.43	1.49	2.13	0.21	4.26	2.55	0.00	0.21	0.00	1.06	14.26
	13.43	2.99	10.45	14.93	1.49	29.85	17.91	0.00	1.49	0.00	7.46	
	13.85	4.35	11.48	20.83	6.67	54.05	26.67	0.00	5.88	0.00	12.82	
CL	15	2	16	0	0	7	2	5	0	30	1	78
	3.19	0.43	3.40	0.00	0.00	1.49	0.43	1.06	0.00	6.38	0.21	16.60
	19.23	2.56	20.51	0.00	0.00	8.97	2.56	6.41	0.00	38.46	1.28	
	23.08	4.35	26.23	0.00	0.00	18.92	4.44	10.87	0.00	58.82	2.56	
CM	1	0	1	22	5	4	3	0	4	0	3	43
	0.21	0.00	0.21	4.68	1.06	0.85	0.64	0.00	0.85	0.00	0.64	9.15
	2.33	0.00	2.33	51.16	11.63	9.30	6.98	0.00	9.30	0.00	6.98	
	1.54	0.00	1.64	45.83	33.33	10.81	6.67	0.00	23.53	0.00	7.69	
DU-L	7	1	3	0	0	1	1	6	0	10	0	29
	1.49	0.21	0.64	0.00	0.00	0.21	0.21	1.28	0.00	2.13	0.00	6.17
	24.14	3.45	10.34	0.00	0.00	3.45	3.45	20.69	0.00	34.48	0.00	
	10.77	2.17	4.92	0.00	0.00	2.70	2.22	13.04	0.00	19.61	0.00	
LC	0	0	11	1	0	1	0	0	1	0	22	36
	0.00	0.00	2.34	0.21	0.00	0.21	0.00	0.00	0.21	0.00	4.68	7.66
	0.00	0.00	30.56	2.78	0.00	2.78	0.00	0.00	2.78	0.00	61.11	
	0.00	0.00	18.03	2.08	0.00	2.70	0.00	0.00	5.88	0.00	56.41	
LL	0	0	22	0	0	0	0	0	1	0	1	24
	0.00	0.00	4.68	0.00	0.00	0.00	0.00	0.00	0.21	0.00	0.21	5.11
	0.00	0.00	91.67	0.00	0.00	0.00	0.00	0.00	4.17	0.00	4.17	
	0.00	0.00	36.07	0.00	0.00	0.00	0.00	0.00	5.88	0.00	2.56	
LM	0	0	0	2	0	0	0	0	10	0	7	19
	0.00	0.00	0.00	0.43	0.00	0.00	0.00	0.00	2.13	0.00	1.49	4.04
	0.00	0.00	0.00	10.53	0.00	0.00	0.00	0.00	52.63	0.00	36.84	
	0.00	0.00	0.00	4.17	0.00	0.00	0.00	0.00	58.82	0.00	17.95	
UC	14	27	0	2	1	1	19	2	0	0	0	66
	2.98	5.74	0.00	0.43	0.21	0.21	4.04	0.43	0.00	0.00	0.00	14.04
	21.21	40.91	0.00	3.03	1.52	1.52	28.79	3.03	0.00	0.00	0.00	
	21.54	58.70	0.00	4.17	6.67	2.70	42.22	4.35	0.00	0.00	0.00	
UL	19	13	1	0	0	1	4	33	0	11	0	82
	4.04	2.77	0.21	0.00	0.00	0.21	0.85	7.02	0.00	2.34	0.00	17.45
	23.17	15.85	1.22	0.00	0.00	1.22	4.88	40.24	0.00	13.41	0.00	
	29.23	28.26	1.64	0.00	0.00	2.70	8.89	71.74	0.00	21.57	0.00	
UM	0	1	0	11	8	2	4	0	0	0	0	26
	0.00	0.21	0.00	2.34	1.70	0.43	0.85	0.00	0.00	0.00	0.00	5.53
	0.00	3.85	0.00	42.31	30.77	7.69	15.38	0.00	0.00	0.00	0.00	
	0.00	2.17	0.00	22.92	53.33	5.41	8.89	0.00	0.00	0.00	0.00	
Total	65	46	61	48	15	37	45	46	17	51	39	470
	13.83	9.79	12.98	10.21	3.19	7.87	9.57	9.79	3.62	10.85	8.30	100.00

SART CLUSTER(Cluster)

Table 6. Cross-classification of ductograms into empirical clusters

women less than 50 years

SART \ CLUSTER(Cluster)	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
CC	7	0	3	5	1	0	2	10	0	0	0	1	3	32
	2.30	0.00	0.99	1.64	0.33	0.00	0.66	3.29	0.00	0.00	0.00	0.33	0.99	10.53
	21.88	0.00	9.38	15.63	3.13	0.00	6.25	31.25	0.00	0.00	0.00	3.13	9.38	
	30.43	0.00	14.29	13.16	6.67	0.00	8.70	34.48	0.00	0.00	0.00	8.33	25.00	
CL	8	19	1	4	0	1	4	0	1	5	0	0	0	43
	2.63	6.25	0.33	1.32	0.00	0.33	1.32	0.00	0.33	1.64	0.00	0.00	0.00	14.14
	18.60	44.19	2.33	9.30	0.00	2.33	9.30	0.00	2.33	11.63	0.00	0.00	0.00	
	34.78	51.35	4.76	10.53	0.00	5.88	17.39	0.00	4.55	10.87	0.00	0.00	0.00	
CM	2	0	0	1	10	1	0	9	0	0	1	2	1	27
	0.66	0.00	0.00	0.33	3.29	0.33	0.00	2.96	0.00	0.00	0.33	0.66	0.33	8.88
	7.41	0.00	0.00	3.70	37.04	3.70	0.00	33.33	0.00	0.00	3.70	7.41	3.70	
	8.70	0.00	0.00	2.63	66.67	5.88	0.00	31.03	0.00	0.00	11.11	16.67	8.33	
DU-L	4	11	0	1	0	0	1	0	0	10	0	0	0	27
	1.32	3.62	0.00	0.33	0.00	0.00	0.33	0.00	0.00	3.29	0.00	0.00	0.00	8.88
	14.81	40.74	0.00	3.70	0.00	0.00	3.70	0.00	0.00	37.04	0.00	0.00	0.00	
	17.39	29.73	0.00	2.63	0.00	0.00	4.35	0.00	0.00	21.74	0.00	0.00	0.00	
LC	0	0	15	0	0	0	1	1	0	0	0	0	3	20
	0.00	0.00	4.93	0.00	0.00	0.00	0.33	0.33	0.00	0.00	0.00	0.00	0.99	6.58
	0.00	0.00	75.00	0.00	0.00	0.00	5.00	5.00	0.00	0.00	0.00	0.00	15.00	
	0.00	0.00	71.43	0.00	0.00	0.00	4.35	3.45	0.00	0.00	0.00	0.00	25.00	
LL	0	1	2	0	0	0	15	0	0	0	1	0	0	19
	0.00	0.33	0.66	0.00	0.00	0.00	4.93	0.00	0.00	0.00	0.33	0.00	0.00	6.25
	0.00	5.26	10.53	0.00	0.00	0.00	78.95	0.00	0.00	0.00	5.26	0.00	0.00	
	0.00	2.70	9.52	0.00	0.00	0.00	65.22	0.00	0.00	0.00	11.11	0.00	0.00	
LM	0	0	0	0	2	0	0	2	0	0	7	0	5	16
	0.00	0.00	0.00	0.00	0.66	0.00	0.00	0.66	0.00	0.00	2.30	0.00	1.64	5.26
	0.00	0.00	0.00	0.00	12.50	0.00	0.00	12.50	0.00	0.00	43.75	0.00	31.25	
	0.00	0.00	0.00	0.00	13.33	0.00	0.00	6.90	0.00	0.00	77.78	0.00	41.67	
UC	0	1	0	16	1	13	0	2	17	1	0	0	0	51
	0.00	0.33	0.00	5.26	0.33	4.28	0.00	0.66	5.59	0.33	0.00	0.00	0.00	16.78
	0.00	1.96	0.00	31.37	1.96	25.49	0.00	3.92	33.33	1.96	0.00	0.00	0.00	
	0.00	2.70	0.00	42.11	6.67	76.47	0.00	6.90	77.27	2.17	0.00	0.00	0.00	
UL	2	5	0	11	0	0	0	0	3	30	0	0	0	51
	0.66	1.64	0.00	3.62	0.00	0.00	0.00	0.00	0.99	9.87	0.00	0.00	0.00	16.78
	3.92	9.80	0.00	21.57	0.00	0.00	0.00	0.00	5.88	58.82	0.00	0.00	0.00	
	8.70	13.51	0.00	28.95	0.00	0.00	0.00	0.00	13.64	65.22	0.00	0.00	0.00	
UM	0	0	0	0	1	2	0	5	1	0	0	9	0	18
	0.00	0.00	0.00	0.00	0.33	0.66	0.00	1.64	0.33	0.00	0.00	2.96	0.00	5.92
	0.00	0.00	0.00	0.00	5.56	11.11	0.00	27.78	5.56	0.00	0.00	50.00	0.00	
	0.00	0.00	0.00	0.00	6.67	11.76	0.00	17.24	4.55	0.00	0.00	75.00	0.00	
Total	23	37	21	38	15	17	23	29	22	46	9	12	12	304
	7.57	12.17	6.91	12.50	4.93	5.59	7.57	9.54	7.24	15.13	2.96	3.95	3.95	100.00

Table 7. Cross-tabulation of pairs of ductograms by Sartorius categories

TABLE OF SART BY SART_1 - DUCTOGRAM PAIRS

SART / SART_1	CC	CL	CM	DU-L	LC	LL	LM	UC	UL	Total
CC	4	0	0	0	0	0	0	0	0	4
CL	12	5	0	0	0	0	0	0	0	17
CM	6	11	2	0	0	0	0	0	0	19
DU-L	1	2	0	5	0	0	0	0	0	8
LC	6	5	7	3	2	0	0	0	0	23
LL	3	5	4	0	3	1	0	0	0	16
LM	5	7	5	0	2	1	1	0	0	21
UC	10	5	6	1	0	5	0	3	0	30
UL	6	9	5	4	3	5	3	8	8	51
UM	1	2	1	1	0	4	2	0	2	13
Total	54	51	30	14	10	16	6	11	10	202

Table 8
Duct Identification/Detached Breasts

Experiment	Ducts Identified	Ducts Cannulated	Ducts Confirmed
1	5	-	-
2	6	-	-
3	3	2	2
4	2	-	-
5	3	2	2
6	3	-	-
7	4	3	3
8	5	1	1
9	7	7	7
10	6	6	6
11	6	6	6
12	8	8	8
13	7	7	7

CHART REVIEW 4000 SERIES - OLIVE VIEW MEDICAL CENTER

Table 9. Chart review

Study #	F	Date	Operation	Diagnosis	Duct ID technique	# Ducts seen	# Ducts cannulated	# Ducts washed	Comments	Cytology	Where sent?	Catheter used
4001	F	08/11/1997	R mast	adeno ca	loupes	2	2	1	1st duct perforated; 2nd duct washed w/saline	ductal epith cells-atyp features: prominent nucleoli, irregular nuclear outlines & overlapping	UCLA	WH double lumen
4002	\	10/22/1997	L mast	ductal ca		1	1	1		deteriorated cells		
4003	A	10/22/1997	R lump	tubular ca, micropap			3	1		deteriorated cells		
4004	A	10/22/1997	L lump	invasive ductal			2	1		deteriorated cells	UCLA	
4005	C	11/19/1997	R lump	abscess	ductoscope		1	1		RBC's, acellular	UCLA	
4006	J	11/19/1997	Bilat mast	Infec-silicone injects			R=2, L=1	R=1, L=0	breast rock-hard due to silicone injections	???		
4007	L	02/05/1998	L lump	fibroadenoma	xenon light, betadyne		1	1		benign epith cells	UCSF	
4008	E	02/05/1998	R lump	fibroadenoma	xenon light, betadyne		1	1		abundant benign epith cells	UCSF	
4009	F	02/18/1998	R re-excis	invasive ductal	blue light	2	2	1	pre, cath, and post	pre/cath-acellular; post-rare cluster well-cohesed duct cells; RBC's	UCLA	
4010	A	02/18/1998	R lump	invasive lob	blue light	5	3	1	5 areas illum noted; NAF at 3 sites	acellular		
4011	H	02/18/1998	L lump	invasive lob	fluorescence				viscous, bloody nipple discharge	mod macphage & rbcs, no duct cells		
		02/18/1998	R breast		fluorescence				able to cannulate where fluor seen-no details or grid			
4012	C	02/20/1998	L mast	adeno ca	blue light		1	1 + naf	Naf washings to Alpaugh	foam cells and ductal cells	UCLA	
4013	H	02/20/1998	R lump	atyp medullay ca	blue light		2	1 + naf	Naf washings to Alpaugh	foam cells, rare ductal cells	UCLA	
4014	F	05/27/1998	R lump	2 palp masses	blue light, fluores		1	1	central duct/area of fluorescence	Package destroyed in mail	NA	
		05/27/1998	L lump	1 mass-fibroad	blue light, fluores		1	1	multiple areas of fluorescence	to Dr. Ljung	NA	
4015	L	06/05/1998	L mast	adeno ca	blue light	2	1	0	unable to asp fluid or collect sm amt bloody fluid			
4016	H	06/10/1998	R lump	fibroadenoma	blue light		1	0	no fluores seen w/ blue light; proc aborted due to pt pain			
4017	C	06/10/1998	L lump	filley defect-ductgrm	blue light	3	1	1	bloody washings and naf	abund iron laden macrophages & partially hemolyzed blood w/ recent bleeding; scant benign epith; poor preserv due to bleeding-not collection or preparation of specimen		
4018	M	06/10/1998	L lump		blue light	2	2	1		benign mamm cells; ben squamous cells prob from skin of nipple	UCSF	
4019	L	06/10/1998	L mast	invasive lobular	blue light		1	0	unable to introduce catheter into duct			
4020	T	06/19/1998	L lump		blue light; goggles	2	0	0	no cann due to blunt tip on cath; no further att- no collection medium brought to OV			black prototype (?)
4021	C	06/19/1998	lump/nodes		blue light; goggles	6	1	0	catheter would slip with pressure			black catheter
4022	C	07/01/1998	lump/nodes	PT. CONSENTED BUT NOTHING DONE					Pt consented only-no proc attempted			
4023	C	09/30/1998	L lump		visual ID only	1	1	1	Dr. Schmit did cannulation/irrigation	abund squam debris, ben epith cells	UCSF	Fuji-1 catheter
4024	F	09/30/1998	R mast	hx L mast- breast ca	visual ID only	1	1	1	Dr. Schmit did cannulation/irrigation	ben epith cells & few anucleated squames	UCSF	Fuji-1 catheter
4025	C	10/21/1998	R mast	dx breast ca-core bx	Zeiss scope	2	2	2	Dr. Schmit did cannulation/irrigation	duct 1-anucleated squames-no epithelial	UCSF	Fuji-1 catheter
										duct 2-histiocytes, ben epith cells-no atyp	UCSF	Fuji-1 catheter
4026	M	11/20/1998	L mast	dx DCIS by FNA	lymphazurin, vis ID	2	1	1	Dr. Schmit & Dr. Hung irrigated w/ lymphaz & saline	atyp epith cells, DCIS intermed - high grade	UCSF	Fuji double lumen
4027	C	11/20/1998	L lump	malig philloides tumor	visual ID only	3	3	3	Dr. Schmit did cannulation/irrigation x 3	mostly benign epithelial cells in all 3 washings	UCSF	Fuji double lumen
4028	L	11/20/1998	L lump w/AND	dx LCIS by excis bx	visual ID only	0	0	0	Dr. Schmit attempted several times to ID/cannulate by probing all over nipple without success		NA	Fuji double lumen

CHART REVIEW 3000 SERIES - UCLA

Study #	F	Date	Operation	Diagnosis	Duct ID technique	# Ducts seen	# Ducts cannulated	# Ducts washed	Comments	Cytology	Where sent?	Catheter used
3000	H	12/12/1996	R mast	dcis comedo	Wood's light		1	1		acellular		double lumen
3001	S	01/06/1997	R mast	no ca	barium	3 +	3	2	see lab notebk for specifics on catheter hookup	ductal cells	UCLA	prototype
			L mast	micropapillary dcis	barium	3 +	3	2	see lab notebk for specifics on catheter hookup	ductal cells	UCLA	prototype
3002	A	01/16/1997	L mast	infiltrat dcis	barium		2	2	see lab notebk for specific pump information	ductal cells	UCLA	
3003	F	01/21/1997	R mast	dcis non-comedo	barium		3	2	nipple became edematous	ductal cells	UCLA	
3004	F	02/12/1997	L mast	dcis non-com; cribform	barium		5	2		ductal cells	UCLA	

3005	02/20/1997	R mast	no ca	barium & suture	1	1	1		acellular	Barsky		
		L mast	invas ductal cis	barium	3	3	1		acellular	Barsky		
3006	07/28/1997	L mast	infiltrat ductal	barium		4	2		acellular, ductal cells	UCLA		
3007	08/28/1997	L mast	infil lob & ductal CA	barium	3	2	1		ductal cells	UCLA		
3008	03/19/1998	R mast	fibrocystic (?)	blue light		1	1	1/2 wash Alpaugh, 1/2 to Ljung & post-can Alpaugh	1 slide from SF: Acellular	LA & SF		
3009	03/25/1998	R prophy mast	atyp	blue light	mult areas of fluorescence; two prominent							
		L mast	dcis	blue light	5	1	0	No NAF on either breast after several attempts	NA	NA		
3010	04/06/1998	R mast	dcis		4 (nipple fluoresced, not cannulated, nipple to Alpaugh)							
		L mast	atyp duct hyperplasia	barium	3	2	1	nipple fluoresced; naf & washings to Alpaugh		Alpaugh		
3011	04/08/1998	L mast	colloid ca	specimen not used for study								
3012	04/24/1998	L mast	infilt duct	no fluores noted	3	3	0	glide wire buckled x 3	NA	NA		
3013	04/29/1998	R mast	dimpling	blue light				unable to detect any area of fluores; no succ cann	NA	NA		
		L mast	lobular	blue light	3	1	0	removed keratin plugs	NA	NA		
3014	05/14/1998	R mast	ductal, infilt lobular	blue light & barium	2	1	1	1 central area of fluores; keratin plug removed	Hirschowitz-acellular;Ljung ben mamm epith cells	LA & SF		
3015	05/19/1998	L mast	dcis & inv dcis			2	0	nipple became edematous	NA	NA		
		R mast	inv lobular	barium		1	1	washings & naf to Ljung (x2)	small & abund clusters ben mamm epith cells	UCSF		
3016	06/24/1998	mrm		Lymphazurin	3	2	0	Gont, Nikolchev, Alpaugh processed detached br	NA	NA		
				black ink used on 1								
3017	06/29/1998	R mast			6	3	0	elderly lady, no fluid. Krupali practiced cann ducts	NO ATTEMPT TO OBTAIN WASHINGS			
3018	07/21/1998	mrm		black ink	7	7 ?		MA notes: very successful, black ink introduced into all 7; identified, cannulated ducts & confirmed via histological analysis		Alpaugh		
3019	10/07/1998	L mast	hx R mast-LCIS	wood's lt, meth blue	5	3	1	probe used to help with cannulation; naf obtained	naf-Montgomery; anucleated squames-no epithel duct-anucleated squames; no epithelial component	UCSF	Fuji	
3020	10/07/1998	L mast	DCIS	wood's lt, meth blue	6	4	2	probe used to help with cannulation	both ducts-anucleated squames-no epith cells	UCSF	Fuji	
3021	11/13/1998	Bilat mast	LCIS & ADH-Lt lumpec	Leica GZ6 & bl dye	3	3	1	more success w/single lumen than double lumen Nothing done with right breast; work on Lt only	anucleated squames; no epithelial component	UCSF	double & single lumen	
3022	11/13/1998	R mast	extensive DCIS	Leica GZ6 & bl dye	2	2	1	exten DCIS, neg mammog, det by lump then bx	3 samples total-#1 anucleated squames; no epith comp. #2 proteinaceous w/few polys & occas epith that appear modertely enlarged - difficult to ID due to fair preservation. #3 abund protein, few polys & histioc, occas epith cell without significant abnormality	UCSF	double & single lumen	
3023	12/08/1998	R mast	invasive ductal ca	Isosulphen blue	3	1	0	used 50/50 conray & bl dye; radiology films taken; no washings collected and edema present		NA	double lumen	
3024	12/17/1998	Bilat mast-used	R only	Lymphazurin	3	2	2	lymph, lidocaine, cytolyt & several diff cann methods		UCSF	double lumen	

Table 10. Cytology results/catheter type

Cytology results by catheter series	# of ducts	type of catheter	unsuccessful*	acellular	ductal cells	degenerate	other cells	total cells
0001-0009	10	ductoscope	2(20%)	1(10%)	5(50%)		1(10%)	6(60%)
1000/2000	34	galac/angio	9(26%)	6(18%)	10(29%)	4(12%)	6(18%)	18(53%)
3000/4000	48	double lumen	2(4%)	3(6%)	28(58%)	9(19%)	4(8%)	37(77%)
*technical difficulties								
3000 (detached)	28	double lumen			13(46%)			18(64%)
4000(attached)	20	double lumen			15(75%)			19(95%)

Table 11. Chilean data

StudyI	*PtID*	*Patient Name*	*Washings cytology*	*Pathology*
15001	64441	Ana Illanes Betroiza	benign ductal	intraductal hyperplasia, fibrocystic, fibroadenoma
15002	92051	Gladys Mardones Pavez	NA	
15003	91975	Virginia Basualto Ayala	benign ductal	fibrocystic, fibroadenoma, intraductal hyperplasia
15004	92546	Mirtha Rodriguez	2 ducts. A: benign ductal B:other	mammary ectasia
15005	92611	Edelmira Olea	benign ductal; other	invasive ductal, moderately differentiated (10%); DCIS (90%),
15006 L	92424 L	Maria C Navarro Tejada	benign ductal; other	fibrocystic, fibroadenoma
15006 R	92424 R	Maria C Navarro Tejada	benign epithelial; other	fibrocystic, fibroadenoma

Appendix B.

THE ANATOMY OF THE BREAST DUCTS. Love SM* Barsky S, Chou J, Offodile R, Alpaugh M, Grosser S, Pesicka D. University of California , Los Angeles.

Although the standard teaching has been that there are 15-20 breast ducts, we have been unable to find an actual study supporting this allegation. In an attempt to describe the anatomy of normal breast ducts, we analyzed 664 ductograms done on 474 women and the pattern of breast duct orifices of 219 lactating women (424 nipples.) Using cluster analysis we found that the median number of lactating orifices identified per breast was 5 with a range of 1-17. 98.8% of women had 13 or less orifices. The pattern of duct orifices was consistent with 4-5 orifices in the center and the rest peripherally arranged. The analysis of ductograms, using one ductogram per woman, resulted in 11 clusters. Again we found 4-5 central ducts which directed back toward the chest wall, accompanied by more peripheral ducts directed radially. Although they were in different patient populations, the nipple duct study and the ductogram studies corroborated a central collection of ductal orifices and corresponding ducts and a more peripheral one-concentric circles rather than radial wedges. We then confirmed these observations in a series of 13 fresh surgically removed breasts by identifying the orifices using a subareolar injection of lymphazurin followed by cannulation of the ducts and injection of tissue specific marking dyes. This confirmed 5-9 non anastomosing ducts per breast which do not correspond to quadrants or radial wedges but project back to the chest wall in a pattern of two concentric circles.

This work was supported by the US Army Medical Research and Materiel Command under DAMD17-96-C-6117

IDENTIFICATION OF PREMALIGNANT AND MALIGNANT BREAST CELLS IN HIGH-RISK WOMEN BY DUCTAL LAVAGE: RESULTS FROM A PILOT STUDY

D. Hung and S. Love
Pro•Duct Health, Inc., Menlo Park, CA.

All breast cancers originate in the single layer of epithelial cells that line the ductal/lobular systems of all breast milk ducts. While these epithelial cells previously have been recovered in nipple aspirate fluid (NAF) using manual breast pumps (Petrakis et al and Sartorius et al), nipple aspiration is a relatively inefficient technique for recovering breast ductal epithelial cells for cytological analysis. On the basis of this earlier work, Pro•Duct Health refined the technique of ductal lavage and developed a microcatheter specifically for this procedure. **Methods:** Twenty-seven (27) ductal lavage cases were conducted under this pilot study. Twenty-one (21) cases were conducted at the Pro•Duct Health facility in Menlo Park, California in women with a previous personal or family history of breast cancer. Six (6) cases were conducted at the National Cancer Institute in Santiago, Chile in women with suspicious mammographic abnormalities who were scheduled for subsequent biopsies. All NAF and ductal lavage samples were cytologically analyzed according to standardized criteria that are virtually identical to the 1997 NCI FNA consensus criteria. Cytological diagnoses were rendered as either insufficient cellular material for diagnosis (ICMD), benign (B), atypical non-suspicious for malignancy (ANSM), atypical suspicious for malignancy (ASM), or malignant (M). Additionally, the cell recovery in 8 of the Pro•Duct Health lavage cases was compared against NAF recovery within the same subject. **Results:** Cannulation with the ductal lavage catheter was attempted on ductal orifices identified by NAF (approximately 1.5 per breast). Successfully cannulated ducts (80% of NAF-yielding ducts) were lavaged with saline or radiographic contrast. The following cytological diagnoses were rendered:

Cytology	Pro•Duct Health	National Cancer Institute
ICMD	1	0
B	18	5
ANSM	2	0
M	0	1

The malignant cytological finding was confirmed by pathological biopsy. Comparative cell quantification data in the 8 NAF/lavage cases demonstrated that the ductal lavage technique allows for greater cell recovery than the NAF technique. In all but 2 cases, the total number of ductal epithelial cells recovered by lavage (>140,000) was greater than that recovered by NAF (~2,000). Six of the 8 lavage samples contained epithelial cell clusters, whereas none of the NAF samples contained such clusters. There were no significant adverse events associated with either the NAF or ductal lavage procedures.

Conclusion: Ductal lavage is an effective method of identifying pre-cancerous and cancerous cells.

Attorney Docket No. 30435
UCLA Case No. 97-097-02
Licensee No. 18612-001010

PATENT APPLICATION

METHODS AND KITS FOR IDENTIFYING DUCTAL ORIFICES IN A NIPPLE

Inventors:

SANFORD H. BARSKY,
a citizen of the United States of America,
residing at 10422 Lindbrook Drive
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SUSAN M. LOVE,
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residing at 16593 Via Floresta
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MARY L. ALPAUGH,
a citizen of the United States of America,
residing at 10792 Wilkens Avenue
Los Angeles, California 90024.

Assignee:

The Regents of the University of California
University of California
1111 Franklin Street, 12th Floor
Oakland, CA 94607-5200
A corporation of California

Status:

Small Entity

Attorney Docket No. 18473U-000300
UCLA Case No. LA98-049

PATENT APPLICATION

METHOD AND KIT FOR OBTAINING FLUIDS AND CELLULAR MATERIAL FROM BREAST DUCTS

Inventor:

SUSAN M. LOVE, a citizen of the United States of America, residing at 16593 Via Floresta Pacific Palisades, California 90272

Assignee:

THE REGENTS OF THE UNIVERSITY OF CALIFORNIA
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Status:

Small Entity

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
(650) 326-2400

FIRST AMENDED AND RESTATED

CERTIFICATE OF INCORPORATION

OF

WINDY HILL TECHNOLOGY, INC.

The undersigned, Julian Nikolchev and Michael W. Hall, hereby certify that:

1. They are the duly elected and acting President and Secretary, respectively, of Windy Hill Technology, Inc., a Delaware corporation.

2. The Certificate of Incorporation of this corporation was originally filed with the Secretary of State of Delaware on May 23, 1997.

3. The Certificate of Incorporation of this corporation shall be amended and restated to read in full as follows:

ARTICLE I

"The name of this corporation is Windy Hill Technology, Inc. (the "Corporation").

ARTICLE II

The address of the Corporation's registered office in the State of Delaware is Corporation Service Company, 1013 Centre Road, Wilmington, Delaware 19805, County of New Castle. The name of its registered agent at such address is Corporation Service Company.

ARTICLE III

The purpose of the Corporation is to engage in any lawful act or activity for which corporations may be organized under the Delaware General Corporation Law.

ARTICLE IV

(A) **Classes of Stock.** The Corporation is authorized to issue two classes of stock to be designated, respectively, "Common Stock" and "Preferred Stock." The total number of shares which the Corporation is authorized to issue is Fifteen Million (15,000,000) shares, each with a par value of $0.0001 per share. Ten Million (10,000,000) shares shall be Common Stock and Five Million (5,000,000) shares shall be Preferred Stock.

(B) **Rights, Preferences and Restrictions of Preferred Stock.** The Preferred Stock authorized by this First Amended and Restated Certificate of Incorporation may be issued from time to time in one or more series. The first series of Preferred Stock shall be designated

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
9200 Corporate Boulevard
Rockville MD 20850

JUN 9 1999

Ms. Angela B. Soito
Regulatory and Quality Affairs Manager
Windy Hill Technology, Inc.
1010 Hamilton Court
Menlo Court, California 94025

Re: K983867
Trade Name: Windy Hill Technology Fuji Catheter
Regulatory Class: II
Product Code: KNW
Dated: April 27, 1999
Received: April 29, 1999

Dear Ms. Soito:

We have reviewed your Section 510(k) notification of intent to market the device referenced above and we have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (Premarket Approval), it may be subject to such additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 895. A substantially equivalent determination assumes compliance with the current Good Manufacturing Practice requirement, as set forth in the Quality System Regulation (QS) for Medical Devices: General regulation (21 CFR Part 820) and that, through periodic (QS) inspections, the Food and Drug Administration (FDA) will verify such assumptions. Failure to comply with the GMP regulation may result in regulatory action. In addition, FDA may publish further announcements concerning your device in the Federal Register. Please note: this response to your premarket notification submission does not affect any obligation you might have under sections 531 through 542 of the Act for devices under the Electronic Product Radiation Control provisions, or other Federal laws or regulations.

This letter will allow you to begin marketing your device as described in your 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801 and additionally 809.10 for in vitro diagnostic devices), please contact the Office of Compliance at (301) 594-4595. Additionally, for questions on the promotion and advertising of your device, please contact the Office of Compliance at (301) 594-4639. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 807.97). Other general information on your responsibilities under the Act may be obtained from the Division of Small Manufacturers Assistance at its toll-free number (800) 638-2041 or (301) 443-6597 or at its internet address "http://www.fda.gov/cdrh/dsmamain.html".

Sincerely yours,

Celia M. Witten, Ph.D., M.D.
Director
Division of General and
Restorative Devices
Office of Device Evaluation
Center for Devices and
Radiological Health

Enclosure

EXHIBIT H:

INDICATIONS FOR USE STATEMENT

510(k) Number (if known): K983867

Device Name: Windy Hill Technology Fuji Catheter

Indications for Use:

The Fuji Catheter is designed to perform contrast enhanced radiography of breast milk ducts. It may also be used for the collection of cells and/or fluid for cytological analysis.

(PLEASE DO NOT WRITE BELOW THIS LINE – CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of Device Evaluation (ODE)

Prescription Use X OR Over-The-Counter Use _____
(Per 21 CFR § 801.109)

(Optional Format 1-2-96)

(Division Sign-Off)
Division of General Restorative Devices
510(k) Number K983867

List of Salaried Personnel

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DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
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REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 2 Feb 01

MEMORANDUM FOR Administrator, Defense Technical Information Center, ATTN: DTIC-OCA, 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statements

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for the following grant numbers:

96-C-6117	ADB233743, ADB243030, ADB259960
94-J-4113	ADB251394, ADB241076, ADB220530

Request the limited distribution statement for Accession Document Numbers listed be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or by email at judy.pawlus@det.amedd.army.mil.

FOR THE COMMANDER:

Phylis Rinehart

PHYLIS M. RINEHART
Deputy Chief of Staff for
Information Management